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STIMULATION OF THE LATERAL HYPOTHALAMUS PRODUCES ANTINOCICEPTION MEDIATED BY 5-HT_{1A}, 5-HT_{1B} AND 5-HT_3 RECEPTORS IN THE RAT SPINAL CORD DORSAL HORN

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Abstract—The lateral hypothalamus is part of an efferent system that modifies pain at the spinal cord dorsal horn, but the mechanisms by which lateral hypothalamus-induced antinociception occur are not fully understood. Previous work has shown that antinociception produced from electrical stimulation of the lateral hypothalamus is mediated in part by spinally projecting 5-hydroxytryptamine (5-HT) neurons in the ventromedial medulla. To further examine the role of the lateral hypothalamus in antinociception, the cholinergic agonist carbamylcholine chloride (125 nmol) was microinjected into the lateral hypothalamus of female Sprague–Dawley rats and nociceptive responses measured on the tail-flick and foot-withdrawal tests. Intrathecal injections of the selective 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 receptor antagonists, WAY 100135, SB-224289, and tropisetron, respectively, and the non-specific antagonist methysergide, were given. Lateral hypothalamus stimulation with carbamylcholine chloride produced significant antinociception that was blocked by WAY 100135, tropisetron, and SB-224289 on both the tail-flick and foot-withdrawal tests. Methysergide was not different from controls on the tail flick test, but increased foot-withdrawal latencies compared with controls. These results suggest that the lateral hypothalamus modifies nociception in part by activating spinally projecting serotonin neurons that act at 5-HT_{1A}, 5-HT_{1B}, and 5-HT_3 receptors in the dorsal horn. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: antagonist, serotonin, nociception.

The lateral hypothalamus (LH) is part of a descending system involved in acute nociceptive modulation in the spinal cord dorsal horn of the rat. For example, electrical stimulation or microinjection of morphine in the LH in awake rats (Dafny et al., 1996; Franco and Prado, 1996) or glutamate in lightly anesthetized rats (Behbehani et al., 1988) produces antinociception on the tail-flick test, while microinjection of carbamylcholine chloride (carbachol) in the LH produces moderate antinociception on the tail-flick and foot-withdrawal tests (Holden and Naleway, 2001; Holden et al., 2002).

The LH appears to modify nociception in part through the nucleus raphe magnus (NRM) in the ventromedial medulla (VMM). The VMM provides much of the serotonin (5-HT) input to the dorsal horn and produces antinociception mediated by 5-HT when activated directly (Dahlstrom and Fuxe, 1965; Satoh et al., 1980; Bowker et al., 1981; Basbaum and Fields, 1984; Jensen and Yaksh, 1984; Hammond et al., 1985; Ruda et al., 1986; Heinricher et al., 1999; Gilbert and Franklin, 2001; Buhler et al., 2004). Efferent projections from the LH to the VMM and, specifically the NRM, have been identified (Berk and Finkelstein, 1982; Schwanzel-Fukuda et al., 1984; Hosoya, 1985), and behavioral studies support the neuroanatomical findings. For example, electrical thresholds in the LH required to induce antinociception are significantly increased following intrathecal injection of methysergide, a non-specific 5-HT receptor antagonist (Aimone and Gebhart, 1987). In addition, inactivation of the VMM by lidocaine injection or ibotenic acid lesion requires a significant increase in focal electrical stimulation in the LH for tail-flick inhibition (Aimone et al., 1988), suggesting a role for the VMM in LH-mediated antinociception. As electrical stimulation can activate fibers of passage as well as neurons, it is not clear whether chemical stimulation of the LH produces antinociception mediated by 5-HT in the spinal cord dorsal horn.

The objective of the present experiments was to determine whether antinociception from activation of neurons in the LH is mediated by serotonergic receptors in the spinal cord dorsal horn. The 5-HT receptors implicated in antinociception include 5-HT_{1A}, 5-HT_{1B} and 5-HT_3 receptor subtypes (Glaum et al., 1988, 1990; Alhaider and Wilcox, 1993; Xu et al., 1994; Laporte et al., 1995; Green et al., 2000) which were the focus of this study. Because previous work has shown that modulation of tail and foot withdrawal latencies can differ, both tail flick and foot withdrawal latencies were measured (Fang and Proudfit, 1996, 1998). Preliminary findings have been published as an abstract (Holden and Naleway, 2002).

EXPERIMENTAL PROCEDURES

The Institutional Animal Care Committee at the University of Illinois at Chicago approved the experimental protocols used in this study. The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 90-23, revised 1978). All efforts were made to minimize animal suffering, reduce the numbers of animals used, and use alternatives to *in vivo* experiments.

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Abbreviations: carbachol, carbamylcholine chloride; DMSO, dimethylsulfoxide; LH, lateral hypothalamus; MEnk, methionine enkephalin neurons; NRM, nucleus raphe magnus; PBS, phosphate-buffered saline; RM, repeated measures; tropisetron, 3-tropanyl-indole-3-carboxylate; VMM, ventromedial medulla; WAY, WAY 100135; 5-HT, 5-hydroxytryptamine, serotonin.

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Three sets of behavioral experiments were done to address the research question. In the first set, carbachol or normal saline was microinjected into the LH using a dose previously determined to provide optimum antinociception in a lightly anesthetized acute preparation (Holden and Naleway, 2001) and nociceptive responses were measured. Carbachol is a nonselective cholinergic receptor agonist that resists the actions of cholinesterase and produces activation of neurons at a number of intracerebral sites (Klamt and Prado, 1991). The LH contains both muscarinic and nicotinic receptors (Segal et al., 1978; Rainbow et al., 1984; Cortes and Palacios, 1986; Spencer et al., 1986). To verify that the actions of carbachol were receptor mediated, one group of rats received pretreatment with atropine sulfate (a M1 and M2 acetylcholine-muscarinic receptor antagonist) two minutes prior to carbachol microinjection. In the second set of experiments, carbachol-induced antinociception was obtained, followed by intrathecal administration of one of the following serotonergic antagonists: methysergide, a non-specific 5-HT receptor antagonist; WAY 100135 (WAY), selective for the 5-HT_{1A} receptor (Cliffe et al., 1993); SB-224289, selective for the 5-HT_{1B} receptor (Selkirk et al., 1998; De Vries et al., 1998); or 3-tropanyl-indole-3-carboxylate (tropisetron), selective for the 5-HT_3 receptor (Donatsch et al., 1984; Richardson et al., 1985; Richardson and Engel, 1986; Glaum et al., 1988, 1990). In the third set of experiments, only the receptor antagonists or saline were given intrathecally to determine whether 5HT receptors were tonically active and to verify that the results obtained in the second experiments were not due to injection of a volume of liquid in the intrathecal space.

Animals

Female Sprague–Dawley rats (250–350 g. Charles River, Portage, MI, USA) were used in this study because our previous work was done in females. To reduce the possibility of estrous cycle influence, rats were randomly assigned to groups and no two rats were taken from the same cage on the same day. One hundred nineteen rats were used for the study as reported and each rat was used only once.

Behavioral tests

To determine the effect of carbachol microinjection in the LH on thermal nociception, the tail-flick latency and the foot-withdrawal tests of nociception were used. Briefly, the tail and the hairy surface of the hind paws were blackened with India ink to facilitate more uniform heating of the skin surface using a noxious thermal stimulus. A focused beam of high intensity light was directed at the dorsal surface of the rat's tail and, immediately afterward, at the lateral aspect of the hairy surface of the hind paws. The time interval between the onset of skin heating and the withdrawal response was measured electronically. In the absence of a response, the skin heating was terminated after 8 s to prevent burning of the skin. Three response latencies were measured in succession at three places on the tail and the average of the three values was defined as the nociceptive response latency. For the foot-withdrawal, a single response latency was measured at one place on the hairy surface of each hind foot. Baseline response latencies of the paws and tail were approximately 2–3 s.

Experiment 1: carbachol, atropine, or saline microinjection in the LH

Each rat was lightly anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and the scalp infused with bupivacaine (0.25%; 0.10 ml), which provides local anesthetic relief for approximately 24 h. Supplemental doses of pentobarbital were given during the 60 min procedure if the rats vocalized or moved without stimulation, but supplementation was rarely required. Immediately after anesthesia, the rats were immobilized in a stereotaxic apparatus, and the midline scalp shaved. Using aseptic technique, a 2 cm incision was made, and the muscle and fascia retracted. A 23-gauge stainless steel guide cannula was lowered into the region of the left LH through a burr hole to a location defined by the following stereotaxic coordinates: −1.5 mm from bregma, lateral +1.7 mm, vertical +1.5 mm, incisor bar set at −2.5 mm. A 30-gauge stainless steel injection cannula was connected to a 10 μl syringe by a length of PE-10 polyethylene tubing filled with either saline or a solution of carbachol (125 nmol in 0.5 μl normal saline). The injection cannula was then inserted into the guide cannula and extended approximately 3 mm beyond the end of the guide cannula. After a baseline latency measurement was taken and recorded, either saline or carbachol was injected into the LH over a 1 min period using an electronic syringe pump. Response latencies were then measured at 5 min intervals for 10 min. A separate group of rats received pretreatment with atropine sulfate (14 nmol in 0.5 μl normal saline) in the LH 2 min prior to carbachol injection. Following testing, animals were overdosed with sodium pentobarbital, decapitated, their brains removed and immersion fixed in a solution of 10% neutral buffered formalin.

Experiment 2: stimulation of the LH followed by intrathecal antagonists

Female Sprague–Dawley rats (Charles River; 275–350 g) were randomly assigned to groups and prepared for microinjection in the LH as described in the previous paragraph. In addition, a 32-gauge intrathecal catheter (Micor, Inc., Allison Park, PA, USA) with a 0.003 in. stylette was inserted through an incision in the cisterna magna and the tip positioned over the lumbar enlargement. The stylette was withdrawn and the catheter attached to a length of PE-50 tubing connected to a 100 μl syringe. All drugs were injected into the spinal cord subarachnoid space in a volume of 15 μl and at a rate of 15 μl/min using an electronic syringe pump. Fifteen microliters of physiological saline or dimethylsulfoxide (DMSO) injected intrathecally was used as a vehicle control. The experiment was conducted as follows: following a baseline latency, carbachol (125 nmol) was microinjected into the LH and tail-flick and foot-withdrawal latencies were determined at 5 and 15 min after injection. One of the following drugs was then immediately injected intrathecally: methysergide maleate (97 nmol), WAY, SB-224289, or tropisetron in one of three concentrations (9.7, 97, or 970 nmol). Tail-flick and foot-withdrawal latencies were measured 5 min after intrathecal infusion and at 5 min intervals for 25 min.

Experiment 3: intrathecal antagonists only

In the final set of experiments, female Sprague–Dawley rats (Charles River; 275–350 g) were lightly anesthetized as described, and prepared with intrathecal cannula placement as in the preceding experiment. The rats in experiment 3 did not receive intracerebral microinjections. Following a baseline latency, one of the four serotonergic antagonists (97 nmol), or saline or DMSO for control, was injected into the spinal cord subarachnoid space in a volume of 15 μl and at a rate of 15 μl/min using an electronic syringe pump. Response latencies were then taken every 5 min for 25 min.

Histology

To determine the position of the microinjection sites relative to the LH, 40-μm transverse brain sections were cut from blocks of tissue that contained the visible injection cannula track using a cryostat microtome. The sections were rinsed in cold phosphate-buffered saline (PBS, 10 mM), mounted on gel-coated slides, stained with 0.05% Neutral Red, dehydrated through a series of alcohols and xylenes and coverslipped. The placement of the microinjection cannula was determined by locating the most ven-